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LABORATORY INVESTIGATION

Interleukin-1β stimulates human mesangial cells to synthesize and release interleukins-6 and -8

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Interleukin-1β stimulates human mesangial cells to synthesize and release interleukins-6 and -8. Interleukin-1β (IL-1β) and tumor necrosis factor (TNF) have been reported to stimulate human mesangial cells (HMC) to proliferate and synthesize eicosanoids. We have examined whether they also induce HMC to release cytokines. In this study we show that both IL-1 and TNF stimulate HMC to release IL-6 and IL-8. Cycling and quiescent HMC were stimulated with various concentrations of either recombinant IL-1β or TNF for 1 to 24 hours. IL-1β at doses as low as 6 pg/ml stimulated mesangial cells to synthesize mRNA for both IL-6 and IL-8 as assessed by Northern analysis; mRNA for tubulin remained constant, which demonstrated a specific increase in mRNA. Secretion of IL-6 and IL-8 into the culture medium increased (4.5 to 18 ng/ml and 4 to 40 ng/ml, respectively) measured by ELISAs. TNF had similar effects but only in high concentrations (> 100 ng/ml). IL-1β did not stimulate cells to proliferate, as measured by ^{3}H thymidine incorporation. TNF caused proliferation but only in concentrations over 100 ng/ml. We conclude that IL-1β is a potent stimulator of human mesangial cell production of IL-6 and IL-8, both of which may influence injury in nephritis. TNF also stimulates mesangial cells but only in pharmacological doses.

The existence of the mesangium in the axial region of glomeruli was first recognized by Zimmermann [1] and characterized by Latta, Maunsbach and Madden [2], who suggested that it provided support for glomerular capillaries. It is now known to have a much more dynamic role that includes the clearance of macromolecules and the control of glomerular filtration rate [3, 4]. The normal mesangium contains two distinct cell types embedded in extracellular matrix. Intrinsic mesangial cells have many of the characteristics of vascular smooth muscle and are presumed to secrete matrix. The remaining cells are resident leucocytes, most of which are probably derived from macrophages. The mesangium in glomerulonephritis is infiltrated with neutrophils and monocytes, intrinsic mesangial cells proliferate and there is increased deposition of matrix. Clearly the relations between these processes are crucial to the proper understanding of nephritis.

Recent studies have emphasized the importance of cytokines and polypeptide growth factors in controlling the intensity of inflammation, and the tendency to scarring [5–7]. Macrophages are an uniquely rich source of these factors [8] and when activated release large amounts of tumor necrosis factor (TNF), interleukins-1, -6 and -8, as well as growth factors such as platelet derived growth factor (PDGF) [9] and transforming growth factor-β [10]. These are part of an integrated network whose complexities are only just beginning to be understood [11]. For example, both TNF and IL-1 activate inflammatory cells and stimulate them to synthesize other cytokines, including IL-8 a chemotactic factor for neutrophils and lymphocytes. This also provokes the release of IL-6 [12, 13] and TGF-β [14] which down-regulate (deactivate) macrophages, and limit the capacity for further TNF synthesis release.

TNF and IL-1 are released from nephritic glomeruli cultured in vitro [15, 16], and studies using anti-cytokine antibodies have established that TNF [17] and IL-1 (Karkar and Rees, manuscript in preparation) modulate the severity of inflammation in the heterologous phase of nephrotoxic serum nephritis. Recently TGF-β has been shown to mediate increased matrix synthesis in antibody-induced mesangial proliferative nephritis in rats [18], and this was down-regulated by treatment with anti-TGF-β antibodies [19]. Infiltrating monocytes are the most obvious source of glomerular cytokines in nephritis, but intrinsic mesangial cells probably produce a limited range of cytokines, albeit in smaller quantities. Cultured rat mesangial cells have been reported to produce TNF, IL-1, IL-6, and GM-CSF [20–25], although the methods used to detect them have not always been specific. Mesangial cells also respond to cytokines; TNF, IL-1 and IL-6 have been reported to stimulate rat mesangial cells to proliferate and to induce a range of other responses, including increased matrix synthesis [26], production of cyclic AMP [27], prostaglandins and an increase in protein kinase activity [28]. There are fewer data on human mesangial cells but they proliferate in response to macrophage purified IL-1 [26], and respond to recombinant IL-1β with increased eicosanoid [29] and oxygen radical production [30].

There are difficulties in determining the relevance of in vitro studies to events occurring in vivo, and these are particularly severe in the case of cytokines and mesangial cells. For example, contamination of cultures with small numbers of macrophages in early passages could suggest spuriously that a

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particular cytokine is produced by mesangial cells; cytokines used in many of the early studies were purified from macrophages and it is now known that such preparations often contain more than one cytokine [31]; many of the assays used to quantify cytokines are less specific than originally believed; and lastly, unrealistically high concentrations of cytokines have often been used.

The present study was designed to investigate the effects of two pro-inflammatory cytokines, TNF and IL-1β, on cultured human mesangial cells. In particular, we studied their effect on the production of IL-8, a powerful chemotactic factor which could be important for attracting neutrophils to the inflamed glomerulus, and IL-6 which is known to have broad modulatory effects on immunity, and inflammation [32, 33]. We have also examined the effect of TNF and IL-1β on mesangial cell proliferation. The results show that human recombinant IL-1β is a powerful stimulant for mesangial cell cytokine synthesis and the TNF has the same effect, but only at high concentrations. Neither TNF nor IL-1β induce mesangial cell proliferation at clinically relevant concentrations, although TNF does at pharmacological doses.

Methods

Reagents

Human recombinant IL-1β (hrIL-1β) was obtained from Dr. Alan Shaw (Glaxo Institute of Molecular Biology) and had a specific activity of 2.5×10^7 U/mg protein using the lymphocyte activation assay [34]. Human recombinant TNF was obtained from Dr. V. Schwendemann (Knoll AG, Ludwigshafen, Germany) and had a specific activity of 9.6×10^6 U/mg using the L929 cell cytotoxicity assay [35]. Both reagents were made in *Escherichia coli* and were free from endotoxin contamination using the Limulus amoebocyte lysate assay. This assay was performed using a test kit (Kabi Vitrim Ltd., Uxbridge, UK) with a limit of detection of 5 pg/ml. The cDNA probes for IL-1β, IL-6 and TNF were also supplied by Dr. Shaw. The cDNA probe for human tubulin a 1.6 Kb insert subcloned into pSP64 plasmid was used as a control [36].

Culture of human mesangial cells

Mesangial cells were cultured as previously described [37]. Briefly, glomeruli were purified from the normal cortex obtained from kidneys removed because of renal cell carcinoma. The cortex was diced and pushed through a set of sieves (150 μ, 250 μ, 106 μ and 63 μ) to yield decapsulated glomeruli with little tubular contamination. The purified glomeruli were treated collagenase Type I (Sigma, Poole, UK) at 1 mg/ml for 20 minutes at 37°C, to loosen cell attachment to the matrix. The resulting glomerular fragments were plated on fibronectin coated, 6-well cluster plates, and grown in RPM1 1640 medium (Gibco Ltd, Paisley, UK) supplemented with 10% fetal calf serum, 2 mM L-glutamine and an insulin, selenium, transferrin growth supplement (25 mg insulin from bovine pancreas, 25 mg human transferrin and 25 μg sodium selenite; Sigma) used in accordance with manufacturer's instructions. Penicillin (100 IU/ml) and streptomycin (100 μg/ml) were also added routinely. All cultures were carried out in humidified air with 5% CO_2 at 37°C. The cells were removed when confluent, using trypsin/EDTA and subcultured in 75 sq cm flasks (Linbro, Flow Labs, Rickmansworth, UK) and later in 150 sq cm flasks. The cells used in these experiments were between passage numbers 4 and 8.

Identification of mesangial cells

The purity of mesangial cell cultures was assessed using markers that we have previously shown to discriminate between various glomerular cell types [37]. Samples of cells were subcultured on fibronectin-coated 13 mm glass coverslips placed in 24-well cluster plates and grown in standard medium. Selected coverslips were removed after 48 hours, washed in phosphate buffered saline (PBS), air dried and fixed in ethanol at 4°C for five minutes. The cells were stained for actin filaments using a specific anti-smooth muscle actin monoclonal antibody (Sigma) and for cytokeratin using a monoclonal antibody (Becton-Dickinson, Oxford, UK). Both monoclonal antibodies were detected using a second layer fluorescein labelled anti-mouse Ig antibody (Dako, High Wycombe, UK). The cells were then washed and mounted in citifluor 95% glycerol (Citifluor, University of London, UK). Other coverslips were used to test for uptake of acetylated low density lipoprotein (Ac. LDL), an endothelial cell marker but which is also taken up by macrophages. These coverslips were incubated with dioctadecyl tetramethyl-indo-carbocyanin perchlorate (Dil) labelled Ac.LDL (Biogenesis, Bournemouth, UK) for four hours. After washing, they were fixed in 1% paraformaldehyde for five minutes at room temperature and examined by ultraviolet microscopy.

The cells were also examined by electron microscopy. A cell suspension was fixed in 2% glutaraldehyde for two hours. After washing in phosphate buffer the cells were osmicated and dehydrated in a series of graded alcohols. Between all solution changes cells were spun at 2000 rpm, the supernatant removed, and the pellet resuspended in the next solution. The final pellet was embedded in Taab resin and polymerized at 60°C overnight. Sections of 1 μm were cut and stained with Toludine blue for observation at light microscopy level. Ultrathin sections of approximately 100 nm, were collected on nickel grids and stained with uranyl acetate and lead citrate, for observation on a Philips CM-10 electron microscope.

Proliferation assays

Cellular proliferation was assessed indirectly by incorporation of tritium (^{3}H) labelled thymidine into newly synthesized DNA. Cells were plated into 96-well flat-bottomed cluster plates at a density of 1×10^4 cells per well, and cultured for 48 hours before incubating in culture medium with 0.25% platelet-poor human plasma (PPP) for 48 hours to make them quiescent. The cells were then incubated for 24 hours with hrIL-1β or hrTNF either in quiescing medium containing 0.25% PPP or in full growth medium containing 10% fetal calf serum. Then they were pulsed with ^{3}H thymidine (1μ Ci/well) and cultured for a further 24 hours. The cells were removed using trypsin/EDTA and harvested onto filters using a titertek cell harvester (Flow Labs, Rickmansworth, UK). Incorporated ^{3}H was measured by placing filters into vials containing scintillation fluid and counting on a liquid scintillation counter (Beckman). The results of three replicates for each concentration of cytokine added were recorded as disintegrations per minute (DPM). This enabled us

to assess the effects of the cytokines both on quiescent and proliferating cells.

Northern analysis

Confluent cells in 150 sq cm tissue culture flasks were quiesced for 48 hours to synchronize the cell cycle, and again the cytokines were added in fresh serum-free medium containing 0.25% PPP or full growth medium. They were harvested at various times (most commonly after 3 hr) in guanidinium thiocyanate solution, and the total cellular RNA was purified by cesium chloride density gradient ultra-centrifugation [38]. Aliquots of 30 μg of total cellular RNA from each mesangial cell sample were run in 1% agarose formaldehyde gels and transferred to Genescreen plus™ (New England Nuclear, Boston, Massachusetts, USA) by capillary blot using 10 × SSC solution (1× SSC = 15 mM trisodium citrate, 150 mM sodium chloride). The blots were pre-hybridized in 50% formamide, 10% dextran sulphate, 1 M sodium chloride, 1% SDS with 200 μg/ml carrier DNA (denatured salmon sperm DNA). The cDNA probes were labelled with ^{32}P CTP using the Klenow DNA polymerase reaction [39]. Blots were hybridized with the labelled probes overnight at 42°C in the same pre-hybridization buffer before being washed twice for 30 minutes at 42°C in 1× SSC with 1% SDS and twice at 42°C for 30 minutes in 0.1× SSC with 1% SDS. The blots were exposed to XAR-5 film (Kodak) at −70°C. Each blot contained a positive control using RNA obtained from lipopolysaccharide (LPS)-stimulated human monocytes and negative control with RNA from unstimulated quiescent or proliferating mesangial cells as appropriate. The amount of monocyte RNA used for Northern blots was reduced to 1 μg because the degree of cytokine gene expression was high, and so hybridization with the tubulin probe was barely visible.

IL-6 and IL-8 assays

The IL-6 was measured using a specific capture ELISA assay kit (Quantikine, R and D systems, Minneapolis, Minnesota, USA) which has linear binding from 30 pg/ml to 2 ng/ml. Preliminary experiments showed a 1:50 dilution of the test samples was needed to ensure the results fell on the linear portion of the standard curve, and so tissue culture supernatants from stimulated and unstimulated mesangial cells were incubated at this dilution. The following IL-6 standards were used: 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.3 pg/ml. To determine if the IL-6 was biologically active, IL-6 was also assayed in selected samples using the IL-6-dependent murine hybridoma cell line, B9 [40].

The IL-8 was assayed using a specific capture ELISA [41]. This assay has linear binding from 0.1 ng/ml to 100 ng/ml of IL-8. Samples were incubated on ELISA plates at 1:20 after preliminary experiments had shown this to be the appropriate dilution. IL-8 standard concentrations were used in every assay and a standard curve drawn from this. The biological activity of IL-8 was assessed using the neutrophil shape change assay for neutrophil chemotactic factors described by Haslett et al [42]. Mesangial cells were stimulated for three hours in serum-free medium containing 10 ng/ml IL-1 before the supernatant was removed, the cells were washed in fresh medium and incubated overnight in fresh serum-free medium without IL-1. In the morning, aliquots of 50 μl of this supernatant or a supernatant from unstimulated mesangial cells were added to tubes containing 2 × 10^5 normal human neutrophils prepared by dextran sedimentation, followed by discontinuous plasma Percoll™ (Pharmacia, UK) density gradient centrifugation [42]; cytospins of these cells contained more than 98% pure neutrophils by May-Giemsa. The neutrophils were fixed after 10 minutes at 37°C by adding 2% glutaraldehyde. Neutrophils exposed to chemotactic factors developed pseudopodic and lamellapodic cytoplasmic extensions, that is, they underwent "shape change". The percentage of cells undergoing shape change was calculated by direct counting and was related to the concentration of chemotactic factor. Polyclonal goat anti-IL-8 antibodies were used to inhibit the shape change as a specificity control.

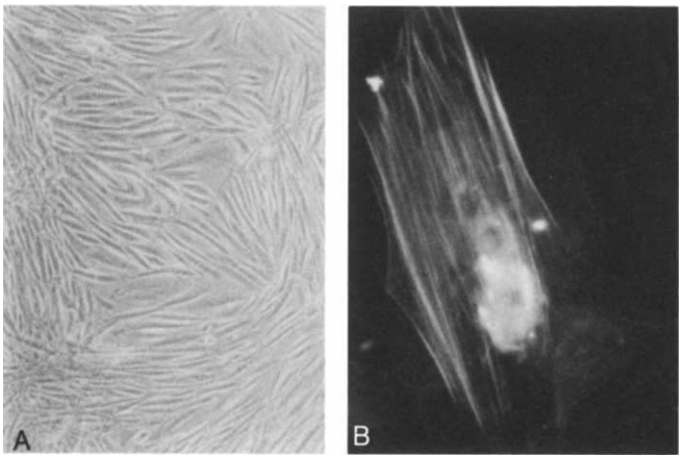

Fig. 1. A. *Light micrograph of confluent human mesangial cells showing elongated cell morphology and multiple layers of cells (×250).* **B.** Human mesangial cells stained with anti-smooth muscle actin antibodies, showing characteristic actin filament arrangement (×500).

Results

Cell culture characteristics

Glomeruli adhered to tissue culture plates after three to four days, cellular outgrowths were visible by five days and reached confluence after six to ten days. The initial outgrowths were heterogeneous, and included cells that bound anti-cytokeratin antibodies and that took up Ac.LDL. After several passages the cultures appeared uniform on light microscopy and consisted of axially elongated cells which resembled smooth muscle cells and grew in swirls. They were not contact inhibited and formed the 'hills and valleys' morphology said to be characteristic of mesangial cells [43, 44] (Fig. 1A). Actin filaments running longitudinally through the cells were seen in preparations stained with anti-smooth muscle actin antibodies, an arrangement typical of mesangial cells [45] (Fig. 1B). Electronmicroscopy showed that the cells had peripheral concentrations of microfilaments, prominant Golgi apparatus and oval nuclei with peripheral heterochromatin (Fig. 2). These features are typical of mesangial cells [44] but not of fibroblasts. None of the cells were stained with anti-cytokeratin antibodies, which identifies epithelial cells, and none took up Ac.LDL. This therefore demonstrates that the cultures were not contaminated with endothelial cells or macrophages.

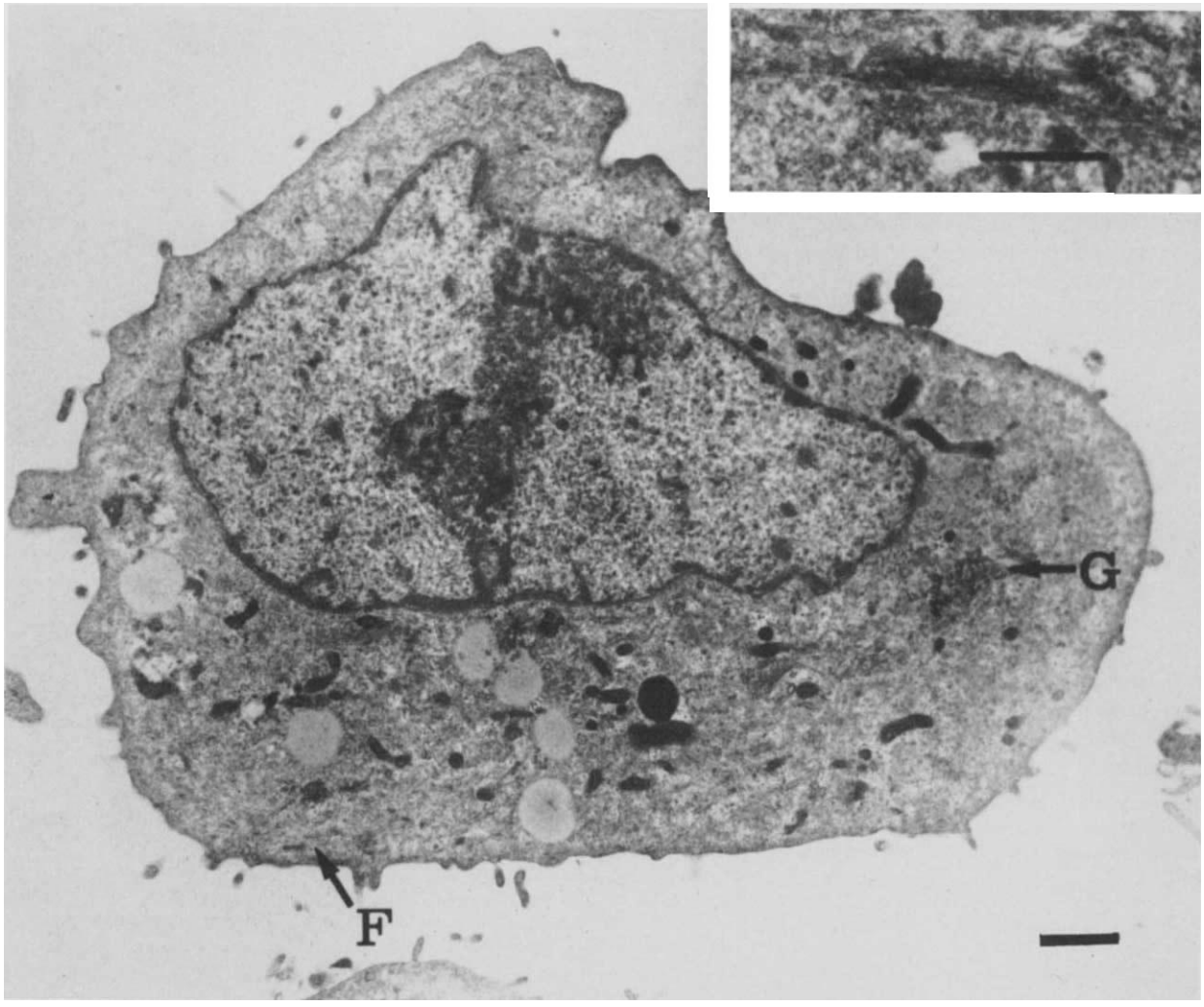

Fig. 2. *Electronmicrograph of a trypsinized mesangial cell showing sparse organelles, abundant microfilaments (F), which in many cells were arranged in plaques (inset), a prominent Golgi apparatus (G) and peripheral nuclear heterochromatin.* In each case the bar represents 1 μm.

Stimulation of mesangial cells by hrIL-1β

Proliferation. In six separate experiments, human recombinant IL-1β in doses up to 1 μg had no effect on mesangial cell morphology or viability when added to quiescent cells growing in medium containing 0.25% PPP, or to cycling cells maintained in full growth medium. IL-1 had equally little effect on incorporation of ^{3}H thymidine by quiescent cells or cycling cells (Fig. 3). Thus, there was no evidence that human recombinant IL-1β was a competence or progression factor for human mesangial cells when studied in this way.

Cytokine mRNA production. RNA was harvested from quiescent and cycling cells, either unstimulated, or incubated for three hours with hrIL-1β in concentrations from 6 pg to 100 ng/ml. The RNA was then subjected to Northern analysis using cDNA probes to tubulin, IL-6 and IL-8 sequentially. Messenger RNA for IL-6 and IL-8 could not be detected either in quiescent or proliferating cells in the absence of IL-1β, but mRNA for both were induced with IL-1β. This effect was found at concentrations as low as 6 pg/ml of IL-1β and appeared to be

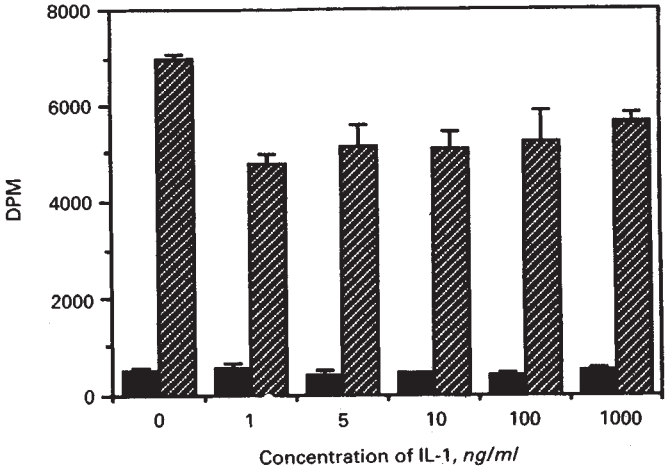

Fig. 3. *Effect of increasing hrIL-1β concentration on proliferation of human mesangial cells, quiescent cells in 0.25% PPP (solid bars) and cycling cells in full growth medium containing 10% fetal bovine serum (hatched bars).* Data from a representative experiment of 6.

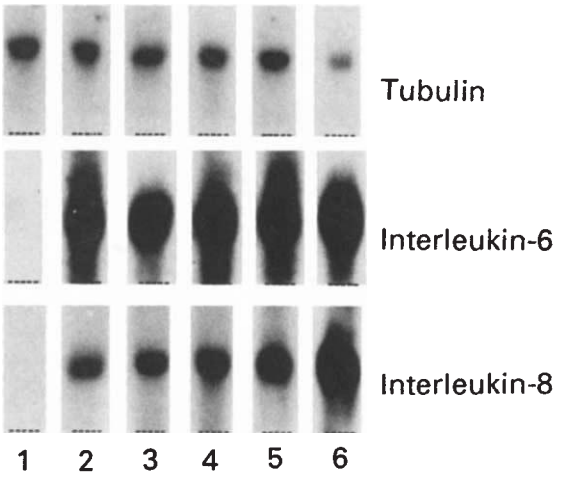

Fig. 4. *Northern blot analysis of quiescent human mesangial cells showing the same blot sequentially probed for tubulin, IL-6 and IL-8.* Cells were unstimulated (1) or stimulated for 3 hours with hrIL-1β 400 pg/ml (2), 1 ng/ml (3), 10 ng/ml (4), 500 ng/ml (5). Lipopolysaccharide (LPS) stimulated human monocyte RNA was used as the control (6).

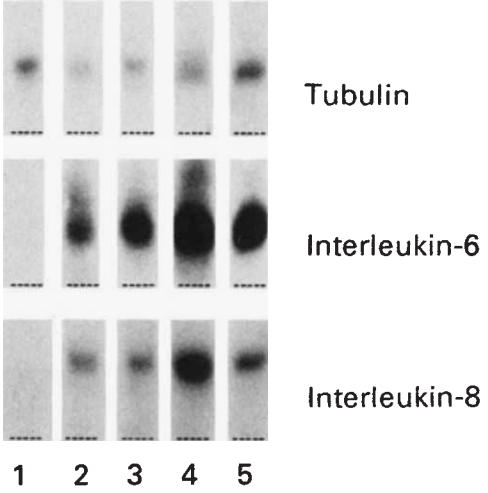

Fig. 5. *Northern blot analysis of quiescent human mesangial cells showing the same blot sequentially probed for tubulin, IL-6 and IL-8.* Cells were unstimulated (1) or stimulated with hrIL-1β 10 ng/ml for 1 hour (2), 2 hours (3), 3 hours (4), 8 hours (5).

greater when higher concentrations were used (Fig. 4). IL-1β did not influence the intensity of hybridization to the tubulin probe. In two time course experiments, IL-6 and IL-8 mRNA concentrations increased after incubation with IL-1β for one hour, reached a maximum after three hours, but was still greater than baseline at eight hours (Fig. 5). There was no qualitative difference in results obtained with quiescent or cycling cells.

The changes in steady state mRNA concentrations correlated with cytokine secretion. Concentrations of IL-6 in supernatants of quiescent unstimulated cells increased approximately fourfold to over 18 ng/ml when the cells were stimulated for three hours with appropriate concentrations of IL-1β (Table 1). The results were very similar for IL-8 and again reflected the changes in steady state mRNA on Northern blots. Unstimulated cells produced in the region of 3 to 4 ng/ml IL-8, which rose to a maximum of 40 ng/ml after hrIL-1β stimulation (Table 1).

Table 1. Effect of IL-1β and TNF on release of IL-6 and IL-8 into culture supernatants

	IL-6	IL-8
	ng/ml	
Untreated	4.5 ± 2.0	3.0 ± 2.0
IL-1β *ng/ml*		
0.006	4.1 ± 2.6	8.4 ± 4.2
0.06	7.0 ± 3.5	12.0 ± 3.1
0.4	13.3 ± 3.1	16.8 ± 5.7
1.0	11.3 ± 4.2	40.0 ± 5.2
6.0	18.3 ± 2.2	40.0 ± 4.9
500	17.0 ± 3.3	36.0 ± 6.1
TNF *ng/ml*		
10.0	5.2 ± 3.5	2.9 ± 1.1
50.0	4.45 ± 3.2	5.4 ± 1.0
100	11.75 ± 2.5	8.27 ± 0.73
1000	12.15 ± 4.0	13.9 ± 0.13

Results are expressed as mean ± standard error.

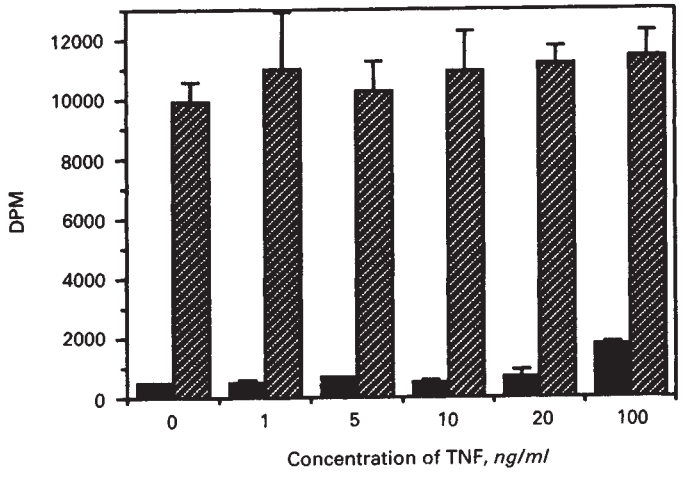

Fig. 6. *Effect of increasing hrTNF concentration on proliferation of human mesangial cells, quiescent cells in 0.25% PPP (solid bars) and cycling cells in full growth medium containing 10% fetal bovine serum (hatched bars).*

Stimulation of mesangial cells by hrTNF

Proliferation. The capacity of TNF to stimulate human mesangial cell proliferation was assessed in five experiments using mesangial cells from different donors. TNF had no effect on mesangial cell proliferation when used at concentrations of 50 ng/ml or less, even though these concentrations have been reported to stimulate fibroblasts, endothelial cells and macrophages [46–50]. However, incubation of mesangial cells with TNF 100 ng/ml and 500 ng/ml caused a three-to fourfold increase in DPM's, suggesting that high concentrations of TNF induce proliferation of mesangial cells (Fig. 6); even so, the increased proliferation is trivial when compared to that observed in full growth medium. TNF neither enhanced nor inhibited proliferation of cells in complete growth medium containing 10% fetal calf serum (Fig. 6).

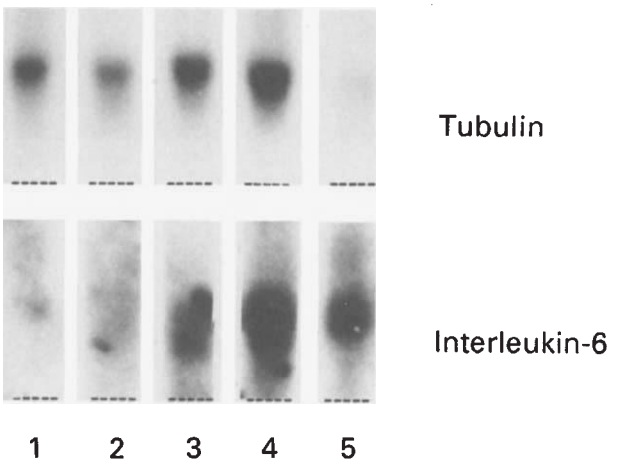

Fig. 7. *Northern blot analysis of quiescent human mesangial cells showing the same blot sequentially probed for tubulin and IL-6.* Cells were unstimulated (1) or stimulated for 3 hours with TNF 10 ng/ml (2), 100 ng/ml (3), 1 μg/ml (4). LPS stimulated human monocyte RNA was used as a control (5).

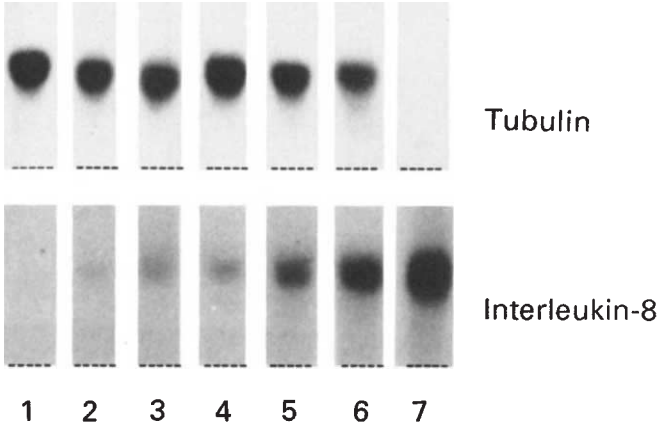

Fig. 8. *Northern blot analysis of quiescent human mesangial cells showing the same blot sequentially probed for tubulin and IL-8.* Cells were unstimulated (1) or stimulated for 3 hours with hrTNF 1 ng/ml (2), 10 ng/ml (3), 50 ng/ml (4), 100 ng/ml (5), 1 μg/ml (6). LPS stimulated human monocyte RNA was used as a control (7).

Steady state mRNA concentrations. The influence of TNF on steady state mRNA concentrations was assessed using both quiescent and proliferating cells. Messenger RNA for IL-6 and IL-8 could not be detected in quiescent cells nor in cells after three hours exposure TNF at concentrations from 1 to 50 ng/ml, but was increased with TNF concentrations similar to those required to induce proliferation; again tubulin gene expression was unaffected. Similar results were obtained in three separate experiments using cells from three different donors. This shows that high concentrations of TNF specifically increase steady state levels of mRNA for IL-6 (Fig. 7) and IL-8 (Fig. 8). The time course was identical to that seen with IL-1β stimulation, and the results were similar in cycling cells. Significant amounts of IL-6 and IL-8 were produced at higher concentrations of TNF and the most effective dose was 1 μg/ml TNF (Table 1).

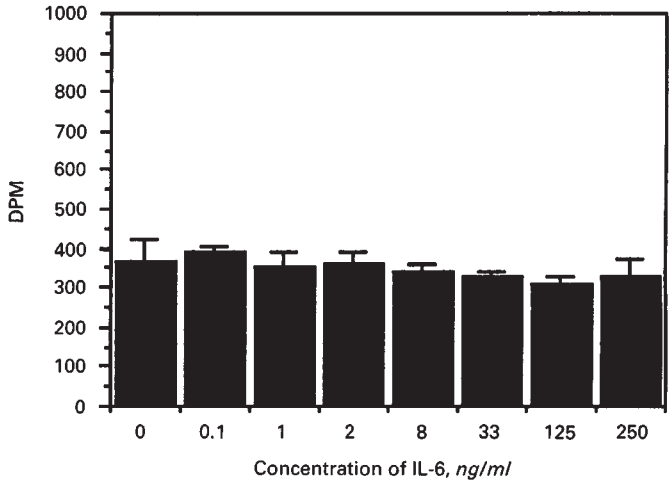

Fig. 9. *Effect of increasing hrIL-6 concentration on proliferation of quiescent human mesangial cells in medium containing 0.25% PPP.*

Mesangial cells and other stimuli

Human recombinant IL-6 in concentrations of 100 pg to 250 ng/ml had no effect on mesangial cell proliferation (Fig. 9). It had equally little effect of mRNA levels of IL-6 and IL-8 in six separate experiments, even though there was strong hybridization with tubulin mRNA. IL-6 at 10 ng/ml and 100 ng/ml did not cause IL-8 release from the cultures. IL-8 was used to stimulate mesangial cells in concentrations ranging from 10 to 20 ng/ml but had no effect.

Bioactivity of IL-6 and IL-8

The activity of mesangial cell-derived IL-6 was assessed from proliferation of the IL-6-specific B9 murine hybridoma cell line. The results show that quiescent human mesangial cells released 1.3 ng/ml of biologically active IL-6, and the concentration increased to 5 ng/ml and 10 ng/ml after stimulation with IL-1β (10 ng/ml) and TNF (1 μg/ml), respectively. Unstimulated cycling cells released 5 ng/ml which increased to 50 ng/ml after exposure to IL-1β.

The ability of mesangial cell supernatants to induce neutrophil shape change was assessed in four separate experiments. Supernatants from unstimulated quiescent cells did not induce shape change, suggesting that they did not contain chemotactic factors. However, supernatants from IL-1β stimulated mesangial cells caused 90% shape change when used neat, and the effect could be diluted out (Fig. 10). The ability of the supernatants to induce shape change was inhibited by a specific goat anti-human IL-8 serum in four separate assays, but not by antibodies to IL-1β. The results of a representative experiment are shown in Figure 10.

Discussion

This study was designed to examine the effects of TNF and IL-1β on human mesangial cells because both cytokines have powerful pro-inflammatory effects and are released in large quantities by activated macrophages [51, 52]. They are found in supernatants from inflamed glomeruli cultured in vitro [15, 16] and could have important influences on the course of nephritis. The effect of both cytokines on synthesis of IL-6 and IL-8 was

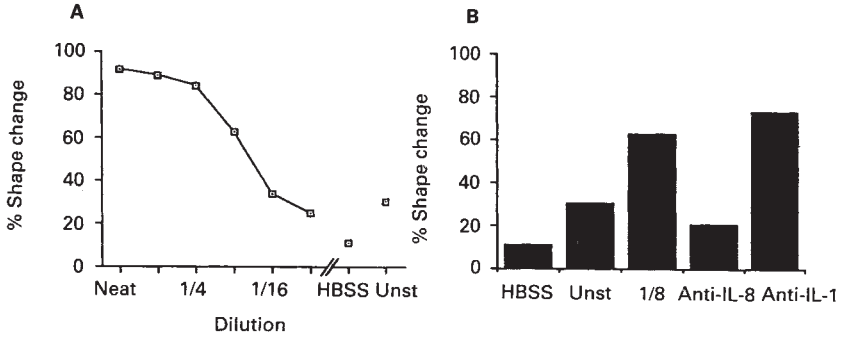

Fig. 10. *Effect of mesangial cell supernatants on neutrophil shape change.* **A.** Dilution curve of supernatant from IL-1 stimulated mesangial cells. **B.** Effect of anti-IL-8 and anti-IL-1 antibodies on neutrophil shape change. (HBSS = Hanks balanced salt solution, Unst = supernatant from unstimulated mesangial cells).

studied. Interleukin-8 was chosen because it is one of a new class of cytokines which are powerful neutrophil chemoattractants and thought to mediate some of the effects of IL-1 and TNF in vivo. Interleukin-6 was studied because of its possible role in a negative feedback loop in controlling the effects of TNF [12, 13].

Interleukin-1β stimulated synthesis of IL-8 by mesangial cells when exposed to as little as 6 pg/ml. Increased steady state mRNA concentrations for IL-8 were found one hour after stimulation and persisted for at least 24 hours. This time course is similar to that of IL-8 mRNA produced by IL-1β stimulated endothelial cells [53, 54] and contrasts with the more transient changes in TNF mRNA [55]. The changes in IL-8 mRNA concentration correspond to its release into the medium which increased tenfold. The amounts released are likely to be biologically important because concentrations as low as 100 pg/ml induce neutrophil chemotaxis and lower concentrations still induce lymphocyte chemotaxis [56]. However, macrophages release much more IL-8 than mesangial cells. At higher concentrations IL-8 causes increased expression of neutrophil CD11b/CD18 complex which mediates neutrophil adhesion [57], the respiratory burst with generation of superoxide and hydrogen peroxide, and exocytosis of neutrophil granules with release of gelatinase, elastase, and β-glucuronidase [58–60], properties with important effects on inflammation.

Interleukin-1β also stimulated IL-6 synthesis with the same time course. Recently Topley et al [29] have shown that low concentrations of hrIL-1β caused human mesangial cells to release eicosanoids. Thus, human mesangial cells are exquisitely sensitive to IL-1β. Previously, IL-1 has been reported to cause proliferation of both rat [61] and human [26] mesangial cells, and so at first sight it is surprising that neither Topley nor ourselves were able to induce mesangial cells proliferation with IL-1β even in concentrations manyfold higher than those needed for cytokine synthesis. However, there are differences between our work and that previously reported. The only human study in which IL-1 was shown to cause mesangial cell proliferation used IL-1 purified from macrophage supernatants [26]. Such preparations of IL-1 are now known to contain substantial amounts of other growth factors, including IL-6 [31] and platelet-derived growth factor (PDGF), which could have been responsible for proliferation rather than the IL-1 itself. However, studies by both Stahl et al [62] and Kester et al [63] have shown that recombinant rat IL-1 can induce proliferation in rat mesangial cells when incubated with low concentrations of serum. Rat mesangial cells have also been reported to release increased amounts of IL-6 when stimulated to proliferate by serum, and this effect is increased by exposure to LPS or TNF. Rat mesangial cells have also been reported to proliferate in response to IL-6 [23, 24] which lead to the suggestion that IL-6 was an autocrine growth factor. Potentially, IL-1β might induce rat mesangial cells to proliferate indirectly through the effects of IL-6 secretion, but experiments to test this have not been reported. We were unable to induce human mesangial to proliferate with hrIL-6 over a wide range of concentrations, either in serum-free medium or in the presence of fetal calf serum. It is too soon to be confident that the different effects of cytokines on human and rat mesangial cell proliferation are due to species variation or to differences in experimental design. We used short proliferation assays to assess the effects of potential growth factors after 48 hours, as these assays have been used previously to demonstrate mesangial cell proliferation induced by serum, PDGF and TNF. In contrast Horii et al [23] and Ruef et al [24] both used longer assays in which DNA synthesis and cell numbers were assessed after 72 hours. This, together with differences in the concentrations of serum used, could explain the discrepancies in the results.

The concentrations of IL-1β required to stimulate mesangial cells are well within the range attainable in vivo, but it is uncertain whether this is true for TNF as concentrations greater than 100 ng/ml were required. Serum TNF concentrations rarely rise above 1 ng/ml even in septicemic patients [64], but local TNF concentrations could be higher, especially adjacent to an activated macrophage. Relatively high concentrations of TNF are also required to stimulate endothelial cells in culture, but macrophages are much more sensitive. Baud et al [27], who showed that high concentrations of TNF stimulated eicosanoid synthesis by rat mesangial cells, have reasoned that glomerular concentrations might reach these levels.

In summary, this paper provides further evidence that IL-1β has powerful effects on human mesangial cells and that these are likely to modulate glomerular inflammation.

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